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Thermolabile Methylenetetrahydrofolate Reductase: An Inherited Risk Factor for Coronary Artery Disease

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Summary

Severe methylenetetrahydrofolate reductase (MTHFR) deficiency with less than 2% of normal enzyme activity is characterized by neurological abnormalities, atherosclerotic changes, and thromboembolism. We have discovered a "new" variant of MTHFR deficiency which is characterized by the absence of neurological abnormalities, an enzyme activity of about 50% of the normal value, and distinctive thermolability under specific conditions of heat inactivation. In this study, lymphocyte MTHFR specific activities in the thermolabile variant and control groups were 5.58 ± 0.91 and 10.33 ± 2.89 nmol formaldehyde formed/mg protein/h, respectively. The difference was significant ($P < .01$). However, there was overlap among the individual values from the two groups. On the other hand, residual MTHFR activity after heat inactivation was 11.2 ± 1.43% in the thermolabile variant and 36.3 ± 5.18% in the controls. There was no overlap. Enzyme studies in 10 subjects with thermolabile MTHFR and their family members support the hypothesis that thermolabile MTHFR is inherited as an autosomal recessive trait. To elucidate the association of thermolabile MTHFR with the development of coronary artery disease, we determined the thermostability of lymphocyte MTHFR in 212 patients with proven coronary artery disease and in 202 controls without clinical evidence of atherosclerotic vascular disease. Thermolabile MTHFR was found in 36 (17.0%) cardiac patients and 10 (5.0%) controls. The difference in incidence between the two groups was statistically significant ($P < .01$). The average age at onset of clinical coronary artery disease in 36 patients with thermolabile MTHFR was 57.3 ± 7.6 years (35–72 years). The mean total plasma homocysteine concentration in patients with thermolabile MTHFR was 13.19 ± 5.32 nmol/ml and was significantly different from the normal mean of 8.50 ± 2.80 nmol/ml ($P < .05$). There was no association between thermolabile MTHFR and other major risk factors. We conclude that thermolabile MTHFR is a variant(s) of MTHFR deficiency which is inherited as an autosomal recessive trait. In addition, it is positively associated with the development of coronary artery disease. Determination of in vitro thermostability of lymphocyte MTHFR is a reliable method for identifying subjects with this abnormality.

Introduction

Increases in serum cholesterol and low-density lipoprotein levels have been identified as major risk factors for the development of atherosclerotic vascular disease (Conference on the Health Effects of Blood Lipids 1979; Castelli et al. 1986; Stamler et al. 1986). Recently, high serum levels of lipoprotein Lp(a) have been shown to be associated with an increased cardiovascular risk (Kostner et al. 1981; Dahlen et al. 1986; Murai et al. 1986). Nonetheless, plasma lipoprotein abnormalities and other well-recognized risk factors do not account for all the cases of atherosclerotic vascular disease (Gordon et al. 1974).

Based on the observation of premature occurrence of arteriosclerosis and of intravascular thromboembolism in patients with severe homocystinuria, homocys-

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t(e)ine has been entertained as an atherogenic factor (McCully 1969). However, even with the available methods for the determination of total homocysteine and protein-bound homocyst(e)ine, it is sometimes difficult to detect individuals with a tendency to develop moderate hyperhomocysteinemia (Mudd et al. 1989). For instance, about one third of the obligate heterozygotes for severe homocystinuria due to cystathionine synthase deficiency have normal plasma protein-bound homocyst(e)ine (Sartorio et al. 1986), suggesting that other factors are significant or also important in influencing plasma homocysteine levels. Even with methionine loading, excessive homocysteine accumulation is not a constant finding in heterozygotes for cystathionine synthase deficiency (Wilcken et al. 1983; Murphy-Chutorian et al. 1985; McGill et al. 1990). Although choline, cyanocobalamin, folic acid, pyridoxine, protein (methionine) intake, female sex hormone(s), and age have been identified as nongenetic factors affecting plasma homocysteine level (Mudd et al. 1989), these factors do not account for all cases of moderate hyperhomocysteinemia. Therefore, a direct investigation of genetic defect(s) which predisposes a subject to the development of moderate hyperhomocysteinemia would be advantageous in the study of atherosclerotic vascular disease.

Recently, we discovered a "new" variant of MTHFR with specific enzyme activity of approximately 50% of the normal mean in lymphocyte extracts. Hyperhomocysteinemia was not a consistent finding in subjects with this variant. The most striking characteristic of this mutant is its in vitro thermolability at 46°C, which provides a clear distinction between this mutant and the normal enzyme found in the majority of the population (Kang et al. 1988*a*, 1988*b*). This new variant was designated "thermolabile variant of MTHFR" and should be distinguished from MTHFR that is thermally unstable at 55°C, which is found in some patients with severe MTHFR deficiency (Rosenblatt and Erbe 1977).

The major clinical features of severe MTHFR deficiency comprise moderate to profound neurological abnormalities, mental retardation, and premature vascular disease (Erbe 1986; Rosenblatt 1989). Biochemical abnormalities include homocystinuria, hyperhomocysteinemia, and sometimes hypomethioninemia. MTHFR activity of cultured fibroblasts from these patients varies from 6% to 12% of the normal mean. The severity of the clinical and biochemical abnormalities appear to be correlated with the degree of enzyme deficiency (Erbe 1986; Rosenblatt 1989). In contrast, these clinical and biochemical features are lacking in subjects with the thermolabile variant of MTHFR, except that increased total plasma homocysteine is observed in some cases. Our preliminary study in 21 cardiac patients under 50 years of age indicated a positive association between this defect and the development of coronary artery disease (Kang et al. 1988*a*, 1988*b*). Thus, we extended this study to a much larger number of subjects with cardiovascular disease irrespective of age.

In this paper, we present evidence supporting the hypothesis that the thermolabile variant of MTHFR is associated with higher plasma homocysteine concentration and the development of atherosclerotic vascular disease. In addition, the inheritance of thermolabile MTHFR follows an autosomal recessive pattern.

Methods

Four hundred and fourteen subjects with or without coronary artery disease were studied. One hundred and ninety-six of 212 cardiac patients were admitted to the Section of Cardiology, Rush-Presbyterian-St. Luke's Medical Center, for the evaluation of cardiovascular disorders. The remaining sixteen cardiac patients were recruited from other institutions. All had extensive evaluation including history, physical examination, blood chemistry, electrocardiogram, chest roentgenogram, and coronary angiography. The majority of the cardiac patients received dipyridamole, diltiazem hydrochloride or nifedipine, and aspirin therapy. Patients were defined as having coronary artery disease when angiograms demonstrated at least 50% obstruction of one or more major coronary arteries. The angiograms were evaluated by two cardiologists (A.S. and N.R.) who were unaware of the results of the biochemical studies. Seventy-two of 202 control subjects were admitted for the evaluation of symptoms potentially related to cardiovascular disease. Investigations which included coronary angiogram showed no evidence of coronary artery disease. All had diagnoses unrelated to atherosclerosis. The rest of the controls were randomly selected from the "normal" population that had neither history nor clinical evidence of cerebrovascular, coronary artery, or peripheral vascular disease.

Among 36 cardiac patients with thermolabile MTHFR, 20 patients and family members of 10 patients agreed to undergo further laboratory studies. In addition, parents of eight patients with severe MTHFR deficiency were available for study. These

eight patients had less than 2% of normal lymphocyte MTHFR activity and their clinical manifestations were characteristic of the severe classical disease. Hence, their biological parents were obligate heterozygotes of the classical mutation but were clinically normal.

Venous blood was placed in ethylenediaminetetraacetate tubes. Lymphocytes were separated immediately with Ficoll-Hypaque (Jondal et al. 1972) and washed with Hank's balanced buffer. Aliquots of lymphocytes were stored at −72°C until the preparation of enzyme extracts. The method for enzyme assay has been described elsewhere (Kutzbuch and Stokstad 1971; Kang et al. 1988*a*, 1988*b*). The reaction mixture contained 0.18 M potassium phosphate buffer pH 6.3, 37 nM menadione bisulfate, 80 μM [^{14}C]methylenetetrahydrofolate (0.8–1.4 × 10^6 cpm), 1.15 mM EDTA pH 7.0, 11.5 mM ascorbic acid, 54 μM flavin adenine dinucleotide, and 258 μl cell lysates in a final volume of 436 μl. Final pH of the reaction mixture was 6.6. Except the blank, all tubes were incubated at 37°C for 20 min. The blank contained all the components of the test sample, but its reaction was terminated at time 0. The reaction was terminated by the addition of 0.3 ml 20 mM dimedone in 1.0 mM potassium acetate buffer pH 4.5. The mixture was placed in boiling water for 5 min, and cooled in ice for 5 min. After the addition of 3 ml toluene, it was vigorously mixed with a Vortex mixer. A 2-ml aliquot of the toluene phase was used for the measurement of radioactivity. Specific enzyme activity was expressed in nmol formaldehyde produced/mg protein/h. It was demonstrated that heat inactivation of MTHFR was more pronounced in the absence of flavin adenine dinucleotide (Rosenblatt and Erbe 1977). Hence, flavin adenine dinucleotide was omitted during heat inactivation. Thermostability of MTHFR was expressed as the percentage of residual activity after heat treatment at 46°C for 5 min. Normal cell lysates retained 20%–50% of the initial enzyme activity after heating. In contrast, lymphocyte extracts with thermolabile MTHFR retained only 7%–17% of the initial activity after heating. Hence, thermolabile MTHFR is defined in this study as that having residual activity of less than 20%.

Total plasma homocysteine was determined as described elsewhere (Kang et al. 1986). The value of total homocysteine was calculated as nmol homocysteine/ml. Other laboratory studies included fasting blood glucose, blood cell counts, serum electrolytes, creatinine, uric acid, total cholesterol, LDL and HDL cholesterol, triglyceride, total protein, albumin, folic acid, and B_{12}.

Quantitative values were expressed as mean ± SD. The differences between the data from the patients with coronary artery disease and the control subjects were evaluated using Student's *t*-test for independent samples. The χ^2 test and Pearson correlation coefficient were used to evaluate the association between two variables. A logistic regression equation relating thermolabile MTHFR to age, sex, diabetes, hypertension, total cholesterol, HDL and LDL cholesterol, triglycerides, total homocysteine, folic acid, cyanocobalamin, and coronary artery disease was developed.

Results

The distribution of residual MTHFR activity after heat treatment at 46°C for 5 min in 202 control subjects is shown in figure 1. The distribution was bimodal and was essentially the same as that found in the cardiac patients in our previous study (Kang et al. 1988*a*). Hence, MTHFR was defined as thermolabile when residual activity was less than 20% after heat inactivation.

The incidence of thermolabile MTHFR in 212 patients with coronary artery disease and 202 controls was compared (table 1). The mean age ± SD in the patients and controls was 60.7 ± 11.7 and 59.6 ± 10.1 years, respectively ($P > .1$) (not shown). Thermolabile MTHFR was found in 36 (17.0%) cardiac patients and in 10 (5.0%) controls. The difference between the two groups was significant ($P < .05$). However, there was no significant difference in the

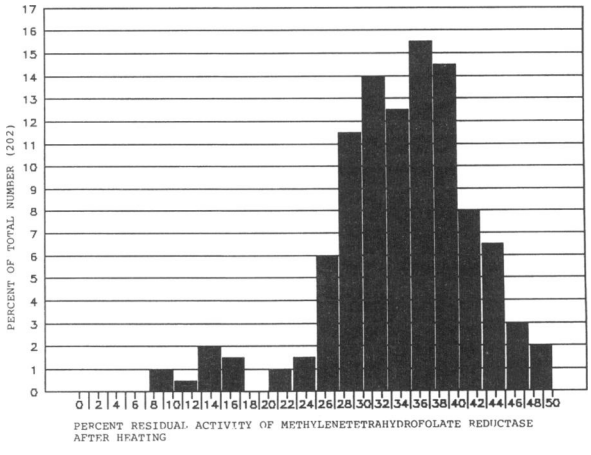

Figure 1 Distribution of residual activity of MTHFR after heat inactivation at 46°C for 5 min in 202 control subjects.

Table 1

Thermolabile MTHFR in Controls and Cardiac Patients

	Controls			Patients		
	Male	Female	Total	Male	Female	Total
No. of subjects studied	124	78	202	147	65	212
No. with thermolabile MTHFR	5	5	10	25	11	36
Percentage with thermolabile MTHFR	4.0	6.4	5.0*	17.0	16.9	17.0*

* $P < .05$ for the difference between the patients and the controls.

incidence of thermolabile MTHFR among the males and females in either group. The average age at onset of clinical coronary artery disease in the 36 cardiac patients with thermolabile MTHFR was 57.3 ± 7.6 years, and the mean age of the 10 subjects with thermolabile MTHFR but without vascular disease was 51.5 ± 16.2 ($P > .05$).

The relationship between residual activity and specific enzyme activity was studied. In 10 cardiac patients with thermolabile MTHFR and their family members (fig. 2), the distribution revealed two distinct groups. Of these 56 subjects, 22 had residual activities in the thermolabile range varying from 8.6% to 17.3%. Thirty-four had thermostable MTHFR with residual activities varying from 24.4% to 45.6%. There was no overlap. Specific enzyme activities in the two groups were 5.58 ± 0.91 and 10.33 ± 2.89 nmol formaldehyde produced/mg protein/h, respectively. The difference in specific activities was significant ($P < .05$). However, there was considerable overlap between the values in the thermolabile and thermostable groups.

The pattern of inheritance in these 10 families was tested by applying the Hardy-Weinberg law (Smith 1956). As shown in table 2, the distribution of thermostable and thermolabile MTHFR among the offspring was tabulated according to the three observed combinations of parental phenotypes. All of the offspring were younger than 40 years of age and were clinically normal. First, in the two families in which both parents had thermolabile MTHFR, all four offspring as expected had thermolabile MTHFR. Second, in the six families in which one of the parents had thermolabile MTHFR, there were 14 offspring with thermostable and five with thermolabile MTHFR. The expected number of offspring with thermostable and thermolabile MTHFR was 15.53 and 3.47, respectively. Third, in the two families in which both parents had thermostable MTHFR, two of seven offspring in one family had thermolabile MTHFR and one of three offspring in the other family had thermolabile MTHFR. The expected total numbers with thermolabile and thermostable MTHFR were 3.25 and 9.75 respectively. The agreement between the observed and the expected numbers was consistent with the hypothesis that thermolabile MTHFR was inherited as an autosomal recessive trait.

Similar to heterozygotes for severe MTHFR deficiency, subjects with the thermolabile MTHFR variant had enzyme activity that was about 50% of the normal mean. Thus, thermostability of MTHFR in

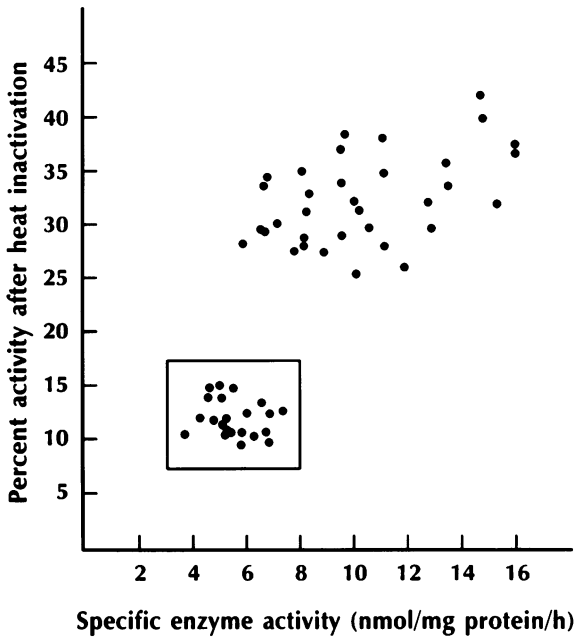

Figure 2 Relationship of MTHFR activity and thermostability in 10 cardiac patients with thermolabile MTHFR and their family members. Specific activity is expressed as nmol formaldehyde produced/mg protein/h. Thermostability is expressed as the percentage of residual activity after heat treatment at 46°C for 5 min. The rectangular box includes 22 subjects with thermolabile MTHFR among 56 individuals.

Table 2

Distribution of Thermostable and Thermolabile MTHFR in Offspring of Parents of Various Phenotypes

Parental Phenotype	No. of Families	No. of Offspring				
		Thermostable MTHFR		Thermolabile MTHFR		
		Expected	Observed	Expected	Observed	Total
Thermolabile MTHFR × thermolabile MTHFR....	2	0	0	4	4	4
Thermostable MTHFR × thermolabile MTHFR....	6	15.53	14	3.47	5	19
Thermostable MTHFR × thermostable MTHFR[a]...	2	9.75	10	3.25	3	13

[a] Both families had offspring with thermolabile MTHFR, indicating that all parents are heterozygotes for thermolabile MTHFR.

heterozygotes for severe MTHFR deficiency was also investigated (table 3). Among 16 "obligate" heterozygotes, parents of eight patients whose lymphocyte MTHFR activities were less than 2.0% of the normal mean, 13 had thermostable MTHFR, whereas three had thermolabile MTHFR. This indicated that alleles for thermolabile MTHFR and for severe MTHFR deficiency were derived from different mutations. The biochemical characteristics of the thermolabile MTHFR in these three heterozygotes were distinguishable from those in the thermolabile variant(s) described in this study and were most likely due to compound heterozygosity for thermolabile MTHFR and severe MTHFR deficiency (Kang et al. 1991).

Some major risk factors for coronary artery disease—diabetes mellitus, hypertension, plasma lipids, and total homocysteine concentrations—were assessed in 37 subjects with thermolabile MTHFR (table 4). These 37 subjects were derived from the cardiac patients, the controls, and their available family members. Among the 20 cardiac patients, two had diabetes, and three had hypertension. They were treated with diet and medication. Serum total, HDL, and LDL cholesterol, serum triglycerides, and total plasma homocysteine in these 20 patients were 218 ± 40 mg/dl, 44 ± 11 mg/dl, 131 ± 29 mg/dl, 218 ± 176 mg/dl and 13.19 ± 5.32 nmol/ml, respectively. In contrast, among the 17 subjects with thermolabile MTHFR but without clinical vascular disease, two had hypertension. Blood chemistry studies in these 17 subjects showed total cholesterol of 226 ± 61 mg/dl, HDL cholesterol of 47 ± 16 mg/dl, LDL cholesterol of 148 ± 54 mg/dl, serum triglycerides of 151 ± 84 mg/dl and total plasma homocysteine of 11.17 ± 3.74 nmol/ml. A logistic analysis showed a significant difference in age and sex ($P < .05$) but no significant difference in lipid values between the two groups. Following the classification provided by the report of the National Cholesterol Education Program Expert Panel (Expert Panel 1988), high total and LDL cholesterol values (total cholesterol >240 mg/dl; LDL cholesterol >160 mg/dl) were seen in 37.8% and 24.3% of the subjects with thermolabile MTHFR with and without cardiac disease, respectively. Nonetheless, the mean total and LDL cholesterol levels were not significantly higher than the normal mean ($P > .1$). The mean total plasma homocysteine levels were 13.19 ± 5.32 nmol/ml in the cardiac patients and 11.17 ± 3.74 nmol/ml in subjects with thermolabile MTHFR but without heart disease (normal value, 8.50 ± 2.80 nmol/ml). Both the cardiac patients and the subjects with thermolabile MTHFR alone had significantly higher homocysteine than normal subjects ($P < .05$). However, there was no significant difference between the cardiac patients and the subjects with thermolabile MTHFR alone. Eight of 17 cardiac patients with normal serum folate and cyanocobalamin (52.9%) had total plasma homocysteine more than 1 SD above the normal mean, suggesting a possible association between thermolabile MTHFR and hyperhomocysteinemia in these patients. Nonetheless, moderate hyperhomocysteinemia was not a consistent finding in these patients. This was

Table 3

Specific Activity and Thermostability of MTHFR in "Obligate" Heterozygotes for Severe MTHFR Deficiency

Family and Parent	Specific Activity[a]	Residual Activity
I:		
Father	5.17	39.6%
Mother	4.35	37.9%
II:		
Father	3.79	24.1%
Mother	5.88	39.0%
III:		
Father	3.22	27.5%
Mother	5.46	46.6%
IV:		
Father	1.91	13.8%
Mother	4.28	43.9%
V:		
Father	4.50	28.8%
Mother	5.69	34.1%
VI:		
Father	10.03	43.2%
Mother	1.76	11.5%
VII:		
Father	8.98	28.3%
Mother	2.34	9.1%
VIII:		
Father	4.47	40.5%
Mother	5.71	34.2%
Heterozygotes (N = 16)	4.85 ± 2.25	31.38 ± 11.8%
	(5.50 ± 1.96)[b]	(35.98 ± 7.1%)[b]
Thermolabile Variant (N = 22)	5.58 ± 0.91	12.1 ± 1.7%
Normal (N = 34)	10.33 ± 2.89	33.3 ± 4.5%

[a] Specific activity of MTHFR in lymphocyte extracts is expressed in nmol formaldehyde formed/mg protein/h.

[b] Values in parentheses are mean ± SD from heterozygotes with thermostable MTHFR.

probably due to an interaction between thermolabile MTHFR and other genetic as well as nongenetic factors affecting homocysteine levels.

Discussion

The observation in our previous study of 21 cardiac patients under 50 years of age (Kang et al. 1988*a*) suggested a positive correlation between thermolabile MTHFR and the development of coronary artery disease. This study of 212 patients with proven coronary artery disease who were recruited without restriction on age or the presence or absence of other major risk factors has confirmed our previous observation.

Thermolability of MTHFR was demonstrated to be the result of an alteration of the enzyme itself and was not due to the effect of an environmental factor(s) (Kang et al. 1988*b*). Although the specific activity of thermolabile MTHFR is about 50% of the normal mean, it is a distinct and different mutation from that of severe MTHFR deficiency. In other words, subjects with thermolabile MTHFR are not heterozygotes for severe MTHFR deficiency. Despite a similar decrease of specific activity, the enzyme in the heterozygotes for severe MTHFR deficiency is thermostable.

Phenotype analysis of subjects with thermolabile MTHFR and their family members supports the hypothesis that thermolability of MTHFR is transmitted

Table 4

Clinical and Laboratory Findings in 37 Subjects with Thermolabile MTHFR

Patient Number	Age	Sex	Diabetes Mellitus	Hypertension	Serum Total Cholesterol (mg/dl)	Serum HDL Cholesterol (mg/dl)	Serum LDL Cholesterol (mg/dl)	Serum Triglyceride (ng/dl)	Plasma Homocysteine (nmol/ml)	Serum Folic Acid (ng/ml)	Serum Cobalamin (pg/ml)
Patients with coronary artery disease:											
1	54	M	No	No	227	41	156	146	13.56	3.0	375
2	51	M	No	No	160	45	99	78	20.80	2.4	436
3	56	M	Yes	No	258	43	190	121	27.24	3.2	376
4	56	M	Yes	No	282	40	173	343	9.16	9.5	432
5	45	M	No	No	199	45	75	393	9.02	6.6	390
6	36	F	No	No	193	22	110	303	11.82	6.1	537
7	69	M	No	No	237	37	148	260	24.30	7.4	420
8	56	M	No	No	207	54	124	146	8.64	3.5	467
9	44	M	No	No	183	50	118	71	13.74	10.5	895
10	62	M	No	No	320	34	111	874	6.72	30.5	699
11	44	M	No	No	172	31	100	204	9.52	26.4	697
12	49	M	No	No	225	54	137	171	12.16	3.9	350
13	46	M	No	No	233	36	167	150	15.38	14.1	356
14	77	F	No	No	211	43	137	157	11.20	6.8	596
15	81	F	No	Yes	210	56	129	123	11.76	12.5	657
16	67	M	No	Yes	243	63	148	158	7.78	6.4	563
17	81	M	No	No	235	58	149	136	11.52	20.0	641
18	76	M	No	Yes	188	55	109	120	13.20	4.7	510
19	69	F	No	No	159	30	96	163	14.40	4.7	483
20	62	M	No	No	224	42	134	238	11.96	4.6	405
Mean ± SD					218 ± 40	44 ± 11	131 ± 29	218 ± 176	13.19 ± 5.32	9.3 ± 7.9	514 ± 145
Subjects without vascular disease:											
1	71	F	No	Yes	264	55	165	219	10.54	4.7	482
2	15	F	No	No	248	68	150	150	6.28	3.7	727
3	42	F	No	No	210	41	136	164	14.60	3.8	411
4	49	F	No	No	301	68	217	72	12.46	3.9	315
5	32	M	No	No	238	33	186	93	11.94	4.9	385
6	45	M	No	No	222	24	159	193	13.12	7.2	513
7	52	M	No	No	258	57	178	115	9.12	7.7	327
8	50	F	No	No	198	51	111	178	7.92	9.2	534
9	57	F	No	No	188	47	107	172	19.60	3.8	318
10	30	F	No	No	149	24	101	121	16.80	1.4	275
11	8	F	No	No	123	23	76	122	4.32	11.7	653
12	25	F	No	No	221	61	149	54	7.62	20.0	546
13	59	F	No	No	115	48	52	74	13.04	5.2	482
14	35	F	No	No	218	83	123	58	10.90	5.0	679
15	70	F	No	Yes	342	36	282	120	10.34	7.9	900
16	69	M	Yes	No	254	44	148	307	10.84	6.7	365
17	66	M	No	No	294	43	179	360	10.46	7.4	678
Mean ± SD					226 ± 61	47 ± 17	148 ± 54	151 ± 84	11.17 ± 3.74	6.7 ± 4.2	505 ± 175

as an autosomal recessive trait, and that subjects with thermolabile MTHFR are mostly homozygotes based on the evaluation of biochemical phenotypes. Molecular evidence of homozygosity is not available at this time.

Clinically, patients with severe MTHFR deficiency had moderate to profound neurological abnormalities and mental retardation (Erbe 1986; Rosenblatt 1989). Postmortem examination in these patients revealed striking vascular lesions, such as thromboembolism in many vessels, occlusions of cerebral, coronary or renal arteries, and infarcts (Kanwar et al. 1976; Baumgartner et al. 1980). The severity of the clinical and biochemical abnormalities appeared to be correlated with the degree of enzyme deficiency. The eight patients with severe clinical disease and severe MTHFR deficiency in the families listed in table 3 had less than 2% of normal mean enzyme activity in lymphocyte extracts (data not shown), whereas patients with the thermolabile MTHFR variant had 50% of normal activity. Hence, it appears that a moderate deficiency of MTHFR activity due to thermolabile MTHFR does not produce neurological abnormalities or mental retardation but may be associated with the development of late-onset vascular disease. Familial hypercholesterolemia is an analogous example of the difference in biochemical severity observed between homozygotes and heterozygotes in the development of premature artherosclerosis (Goldstein and Brown 1989). The clinical picture in homozygotes is remarkably uniform and distinctly different from that in heterozygotes. Unlike the severe disease in homozygotes, the disease in the heterozygotes for familial hypercholesterolemia is much more variable. Similarly, in this study, 21.3% of subjects with thermolabile MTHFR had no evidence of clinical vascular disease even after 50 years of age (table 4). It is suggested that contributions of other factors may also be important in the development of atherosclerotic vascular changes in subjects with thermolabile MTHFR.

It has been documented that severe hyperhomocysteinemia is associated with an accelerated development of atherosclerosis (Gibson et al. 1964; McCully 1969). Subsequently, moderate hyperhomocysteinemia has also been found to be correlated with the occurrence of late-onset vascular disease (Wilcken and Wilcken 1976; Brattstrom et al. 1984; Boers et al. 1985; Murphy-Chutorian et al. 1985, Kang et al. 1986; Malinow et al. 1989). Thus, the elevated plasma homocysteine in most subjects with thermolabile MTHFR suggests that persistent hyperhomocysteinemia is a cause of atherosclerosis. The fact that moderate hyperhomocysteinemia is produced or prevented by various nongenetic factors explains the failure to detect hyperhomocysteinemia in some subjects with thermolabile MTHFR. In other words, reduced activity in thermolabile MTHFR may increase the susceptibility to producing hyperhomocysteinemia because of its interaction with a nongenetic factor(s) such as a low level of serum folate (Kang et al. 1987; Mudd et al. 1989). It has been observed that heterozygosity for severe hyperhomocysteinemia due to cystathionine synthase deficiency is not necessarily associated with elevated plasma homocysteine (Wilcken et al. 1983; Murphy-Chutorian 1985; Sartorio et al. 1986; McGill et al. 1990). However, the possibility that mechanism(s) other than hyperhomocysteinemia causes vascular damage in the subjects with thermolabile MTHFR cannot be excluded. For instance, it may be speculated that, despite a normal homocysteine concentration, these subjects may have decreased transmethylation due to a reduced pool size of methyltetrahydrofolate, which is produced by the action of MTHFR. Pathogenesis of accelerated atherosclerosis due to moderate MTHFR deficiency cannot be fully addressed at the present time.

The present study has demonstrated that the incidence of thermolabile MTHFR is 5% in the controls. This suggests that the gene frequency is very high among the general population. At present, there is no direct evidence that thermolabile MTHFR is derived from a single genotype. Moderate to intermediate hyperhomocysteinemia found in some subjects with thermolabile MTHFR was corrected by oral folic acid supplement (Kang et al. 1987, 1988*b*). Whether hyperhomocysteinemia is induced or not, the effect of decreased activity of MTHFR can be overcome by the supplementation of excess substrate. Hence, the use of folic acid may be effective for preventing hyperhomocysteinemia and the development of atherosclerosis due to thermolabile MTHFR.

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